MODULE 3 - LABORATORY TESTING

Objective

This module is for INFORMATION ONLY. No BETC test questions are from this module.

On completion of this module, participants will be able to:

- Identify the types of indicator microorganisms
- Define Aerobic plate count or Standard plate count

Indicator organisms

Very often microbiologists test for indicator organisms as a substitute for testing for pathogens. The ideal indicator organism should be present when pathogens are present, absent when there are no pathogens, occur in greater numbers than the pathogens to provide a safety margin, and be easy to detect.

Indicator Organisms: Fecal coliform

Staphylococci

Geotrichum candidum

One group of indicator organisms is called the coliform group. Members of this group that grow at elevated temperature are called fecal coliforms. These organisms are found in the gastrointestinal tract of humans and warm blooded animals and have been used as an indicator of human fecal pollution in shellfish and their growing waters, as well as other food commodities. Other examples of indicator groups include staphylococci as an indicator of handling abuse, and Geotrichum *candidum*, the machinery mold, as an indicator of plant insanitation and contaminated equipment.

In the Laboratory

In the laboratory, the microbiologist isolates and identifies the bacteria present. There are specific steps that must be taken to do this. Generally, there are three steps in detecting and identifying bacteria:

Enrichment

Selective agar

Biochemical tests

An enrichment medium is used to favor the growth of the organism you are looking for and to give it a chance to increase in number. You enrich the sample by placing the food product in a medium that has the nutrients discussed earlier that are specific to the type of organism you are trying to isolate.

Then, you place a portion of the enrichment into a medium that selects for the desired organism. This medium contains some of the control mechanisms that were mentioned above (salt, an adjusted pH, or other chemicals or antibiotics) which will select for the organism you want, and not allow quite so many other organisms to grow.

You then streak the selective medium onto an agar plate to isolate a pure colony, one that grew from a single cell. This pure colony is essential for subsequent tests since you need to deal with one organism at a time.

The next step is to subject the isolated colony to biochemical tests that are specific to the type of organism you are looking for and that will confirm that you have the organism you think you do. That is the conventional method of looking for organisms. Each step requires time so it takes a while - usually several days - to get results from the laboratory.

Aerobic Plate Count

You also may hear references to "Aerobic Plate Count", also called "Standard Plate Count". This method provides an estimate of the total number of viable aerobic bacteria in a food, rather than a specific organism. It is generally used to determine food quality. In milk products, high counts may indicate that the milk was handled under insanitary conditions. This procedure is based on the assumption that each microbial cell in a sample will form a visible separate colony when mixed with an agar medium and permitted to grow. The food is diluted and placed in the agar medium in petri dishes so that the colonies can be counted. Microbial populations are at best an estimate. They are reported as Colony Forming Units or CFU per gram.

Most Probable Number (MPN)

Another approach to counting bacteria is a statistical method based on probability theory called the "Most probable number" or "MPN". The test

material is diluted in a series of dilutions to reach a point where not even a single cell remains in the final dilution. If bacteria are present, the medium in the tube is cloudy, or positive. If no bacteria are present, the medium is clear. The pattern of positive and negative tubes at the different dilutions is used to estimate the concentration of bacteria in the original sample. The microbiologist compares the observed pattern of results with a table of statistical values.

Rapid Methods

Some people are surprised that conventional methods take so long. They think everything is high tech now, providing instant results. Not so, traditional methods are still very much in use. However, it is true that recent advances in biotechnology have dramatically altered the diagnostic procedures used in microbiology. These new "rapid methods" provide simpler and often more sensitive and rapid detection of pathogens and their toxins.

The term "Rapid Method" describes a large variety of detection and identification tests, including those that take a few minutes to perform to those that require days. Basically "rapid" means they're faster than conventional microbiological methods.

The use of rapid methods in foods has some limitations. Foods are so complex, and each one's different. Proteins, fats, oils, and other factors can interfere with the tests. The normal bacteria in a food can also interfere with how well a test works. Low numbers of pathogens in foods are hard to detect. Processing of the food changes the bacterial flora and composition of foods. Most of these problems can be remedied by enriching the sample but that takes time which means it's not as rapid.

Each method must be fully evaluated before it can be applied to food testing. There is a process for doing that but even so the rapid methods that are approved can be used only for presumptive screening of foods, a negative result stands but a positive result must be confirmed using standard methods. The Bacteriological Analytical Manual (BAM) contains all the laboratory methods used by FDA in isolating bacteria from foods.

If you want to *detect* bacteria, rapid methods can only be used after the food sample has been through cultural enrichment. If you want to *identify* bacteria, rapid methods are used only after a pure culture isolate has been obtained from the sample.

Types of Rapid Methods

One type of rapid method is a "**miniaturized biochemical identification**" device. They are disposable devices that perform 15 to 24 biochemical tests at one time. They are designed to identify specific bacterial species. The microbiologist must work with a pure culture. Some provide results in 4 hours; most within 24 hours. These units simplify the conventional procedure by eliminating tubes and plate media.

Other rapid method kits speed up standard microbiological methods by using special substrates, enzymes or other apparatus. For example, a **Petrifilm plate count card** contains prepared media. You just add your sample at the appropriate dilution and incubate it. You can then count the bacteria present in the sample. It is disposable and eliminates the need to make the agar plates we talked about earlier.

With a positive **MUG test** kit a special chemical reaction alerts the microbiologist that the organism he's looking for is present. One type of MUG test kit is called a Colicomplete test. The discs are impregnated with two chemicals that react in the presence of coliforms and *E. coli*. You inoculate the tube, add one of the discs and incubate. If a blue color develops you have a presumptive positive for coliforms. You then shine a UV light on the tube. If the tube fluoresces you have a presumptive positive for *E. coli*.

Some of the rapid methods involve using antibodies, nucleic acids or robotics to detect pathogens and toxins. Of these, the antibodies are most versatile and are used in various test kits. They take advantage of antibody-antigen interactions that are specific to a particular pathogen. A latex agglutination test works that way. If the reaction is positive, the latex beads cause the bacteria to clump.

The **ELISA test stands for "enzyme linked immunosorbent assay"**. It is another test that relies on antibody antigen interaction. The final result shows as a color change that can be easily read by the microbiologist. ELISA tests can be used to detect and quantify pathogens and toxins.

A system called **Polymerase Chain Reaction or PCR** uses an enzyme to replicate a portion of a target pathogen's DNA. The reaction involves attaching a marker to the DNA so that it is easily detected. The advantage of this test is that you can detect very small numbers of a particular pathogen. Unfortunately, it does not differentiate between live and dead pathogens.

These are only a few examples of what rapid method kits are and can do. The selection of a rapid method test kits depends in part on the organism

of concern the food product being tested and the intended purpose of the test.

References modules 1, 2, and 3

Ward, D.R. 1997. "Basic Food Microbiology", Food Microbiological Control. FDA.

Feng, Peter. 1997. "Rapid Methods", Food Microbiological Control. FDA